Relative warp and correlation analysis based on distances of the morphological shell shape patterns of *Pomacea canaliculata* Lamarck from Japan and the Philippines

[1]Carlo Stephen O. Moneva, [1]Mark Anthony J. Torres, [2]Takashi Wada, [3]Ravindra Joshi, [1]Cesar G. Demayo

[1] Department of Biological Sciences, College of Science and Mathematics; MSU-Iligan Institute of Technology, Andres Bonifacio Avenue, Tibanga, Iligan City, Lanao del Norte, Philippines; [2] Kyushu-Okinawa National Agricultural Research Center, Koshi, Kumamoto, Japan; [3] College of Agriculture, Forestry and Fisheries, Fiji National University, Koronivia Republic of Fiji.

Abstract. The Golden Apple Snail (GAS), *Pomacea canaliculata* Lamarck is considered one of the serious agricultural pests of rice in Asia. It is being argued that rapid invasion of this species in many variable habitats suggests genetic variability and differentiation which could be expressed at the level of the phenotype. It is therefore the major objective of the study to explore possible phenotypic differentiation in the organism especially in the shape of the shell using geometric morphometric (GM) analysis. Specifically, this study aimed to determine conchological variation in populations of GAS in the Philippines and Japan. Three aspects of the shell shape were studied, which includes the ventral/aperture, dorsal and the top/whorl portion of the shell using correlation analysis based on distances (CORIANDIS). CORIANDIS was used in order to visualize congruence of multivariate traits among *P. canaliculata* populations. The results showed that *P. canaliculata* shell varies in shape and variability may signify distinctive genotypes or adaptation to varying environments exhibited by Japan and the Philippines.

Key Words: CORIANDIS, choncological variation, geographic variation, geometric morphometric analysis, *Pomacea canaliculata*.

Corresponding Author: C. S. O. Moneva, carlox_xia@yahoo.com

Introduction

Pomacea canaliculata Lamarck, popularly referred to as the "golden apple snail", was introduced in the Philippines from the United States in 1980 as an aquarium novelty (Guerrero 2002), for commercial production and as dietary protein supplement in rural areas (Dong *et al* 2011). However, it has been reported that golden apple snails were introduced without prior studies on market information and ecological impact (Teo 2004; Dong *et al* 2011). To date, golden apple snail was recognized as a serious pest having the attributes of fast growth, phenomenal reproductive capacity and a voracious appetite for vegetation (Baldia 1989). Aside from rice seedlings, it also attack wide range of plants including ornamental plants, algae, azolla, duck weed, water hyacinth, corn, citrus, ramie and other succulent leafy plant (Dong *et al* 2011; Baldia 1989). In the Philippines, golden apple snail infested area expanded rapidly from 9500 ha in 1986 to 426,000 ha in 1988 to over 500,00 ha by 1989 and more than 800,000 ha in 1995 (Cowie 2006; Cagauan & Joshi 2003; Joshi *et al* 2005; Joshi 2005). In 1997, Pagulayan documented that apart from being an agricultural pest, the golden apple snail has also replaced the native freshwater snail, *Pila conica*, in Luzon (Guerrero 2002). In Japan, *Pomacea* apple snail was introduced as an edible snail from Taiwan, in 1981 (Mochida 1991). After that, juveniles were sometimes introduced directly from Argentina (Miyazaki 1985).

Growth and reproduction of golden snails were not the same from different regions. The review by Dong *et al* (2011) indicated that golden apple snail in the Philippines behaved in a similar way with the snails in Sabah, but in Japan and Argentina, the snails took a longer time to reach maturity and hatching success being lower with fewer eggs in each cluster. Therefore, allopatric populations inhabiting different habitats may show ecomorphological variations and a questionable species status (Alonso *et al* 2006). It was suggested that the apple snails had a high adaptability to the new environment and was easier to form a new populations (Dong *et al* 2011). It is argued that rapid invasion of this species in many variable habitats suggests genetic variability and differentiation which could be expressed at the level of the phenotype or genotype (Tabugo *et al* 2010). With this background, the primary aim of the study was to analyze and understand the shell shape patterns of *Pomacea canaliculata* collected from agricultural and freshwater ecosystems found in Lanao del Norte and Misamis Occidental, Philippines and in Kikuchi and Okishi, Japan. This research was carried out to detect variations on shell shapes through geometric morphometry by means of Relative Warp Analysis. Correlation Analysis (CORIANDIS) based on distances was also used to better understand variability based on landmark data (Marquez & Knowles 2007). This information could be useful as a preliminary approach to conduct proper agricultural management and regulation with regards to the corresponding species involved.

Material and Method

The golden apple snails (*Pomacea canaliculata*) were obtained from Lanao del Norte (Lala and Kapatagan) and Misamis Occidental (Ozamis City and Plaridel). Samples from Kikuchi and Okishi, Japan were also used. A total of 240 specimens were utilized comprising of 30 females and 30 males for each population. Map showing the study area is shown in Figure 1. Shells were photographed by a digital camera. Images of the shell will always be in the same position with the columella at 90^0 of the x-axis in an aperture view or in the orientation in which the apex is visible. Obtained images were then subject to geometric morphometric methods. The shell of the apple snail is spherical or heliciform or elongate ovate shell form having three to five sutures with wide oval or circular aperture. It has no siphonal canal and the outer lip of the aperture is not reflected. Digital images (ventral, dorsal and top view) were taken for each sample using a standardized procedure (Fig. 2).

Shell shape was studied using a landmark-based methodology that eliminates the effect of variation in the location, orientation, and scale of the specimens. Twenty one anatomical landmarks located along the outline of the ventral/apertural (Fig. 2a) portion of the shell and seventeen anatomical landmarks along the dorsal (Fig. 2b) portion of shell were defined and used. Obtained digitized images of the snails' shell were then outlined with sample points around its contour in order to get the x and y coordinates. This was made possible using an image analysis and processing software Tps Dig freeware 2.12. Tps Dig facilitates the statistical analysis of landmark data in morphometrics by making it easier to collect and maintain landmark data from digitized images (Rohlf 2008a).

These coordinates were then transferred to Microsoft Excel application for organization of the data into groups (based on species). The two-dimensional coordinates of these landmarks were determined for each shell specimen. Then the generalized orthogonal least squares Procrustes average configuration of landmarks was computed using the generalized Procustes Analysis (GPA) superimposition method. GPA was performed using the software tpsRelw, ver. 1.46 (Rohlf 2008b). After GPA, the relative warps (RWs, which are the principal components of the covariance matrix of the partial warp scores) were computed using the unit centroid size as the alignment-scaling method. Histogram and box plots were generated using PAST software (Hammer *et al* 2001) from the relative warps of the shell shapes. Histogram and box plots are a powerful display for comparing distributions. They provide a compact view of where the data are centered and how they are distributed over the range of the variable. Kruskal-Wallis test was used to analyze whether or not the species differ significantly with regards to its shell shape (Demayo *et al* 2011). Canonical Variance Analysis (CVA) was also used in order to compare patterns of population variation. The top or whorl (Fig. 2c) portion of the shell were outlined with 199 outline points using tpsDig program and the tps curve outline was converted to landmarks (corresponding x and y) using tpsUtil ver. 1.44 (Rohlf 2009). The collective coordinates of all individuals were then subjected to Principal Component Analysis (PCA) using geometric morphometric computer application Paleontological Statistics (PAST) version 1.91 software developed by Hammer *et al* (2001). PCA was used to summarize the information of the variations and mean shapes contained in the coefficients of landmark descriptors.

Landmark data obtained for the three shell characters were analysed using Coriandis: Correlation analysis based on distances version 1.1 beta (Marquez & Knowles 2007). This was used to determine associations among multivariate datasets, projections on compromise space, trait variance or disparity, congruence and multivariate covariance measure on how similar the interspecific locations of the three shell characters of golden apple snails. The software was used to determine associations between species of freshwater snails as defined by different multivariate data. The option "Projections on compromise space" was selected, this was done such that all specimens/groups and traits/sets are plotted in the same space, obtained by projecting each dataset plus their weighter average ('compromise') onto the compromise space. Then, the squared distances of each group to the origin are computed for each of the shape data sets, and plotted in a stacked bar graph to give an overall impression of the differences between the three species of freshwater snails (Tabugo *et al* 2010 and in dragonfly wings (Gutierrez *et al* 2011). After that, the scores obtained in the analysis are used in Cluster analysis.

Results and Discussion

Canonical Variance Analysis (CVA) scatter plots of the pooled individuals from different populations of the golden apple snails showed patterns of geographical variation (Fig. 3). Results of the Multivariate Analysis of Variance (MANOVA) (Table 1) for the ventral/aperture portion of the shell obtained p-values of $6.425E^{-22}$ and $2.768E^{-22}$ for female and male populations of golden apple snails respectively. For the dorsal shell portion, MANOVA showed p-values of $1.00E^{-13}$ for female population and $1.561E^{-12}$ for the male population of *P. canaliculata* from varying regions.The summary of the geometric morphometric analysis showing the consensus morphology and variation in the ventral/apertural shell shape pattern of different *P. canaliculata* populations as produced by the relative warps (RW) is shown in Figure 4 and described in Table 3. Projections on the left side of the histogram are considered to be variations in shell shape foreseen as negative deviations of the mean in the axis of the relative warps. Then, on the right side are variations in shell shape foreseen as positive deviations of the mean in the axis of the relative warps. The topmost figure is the mean shape of the samples obtained. Differences between sexes and geographical regions are based on the results of the Kruskal-Wallis test shown in Tables 2 (ventral/aperture) and 4 (dorsal).

For the dorsal part of the shell, Relative warp analysis obtained four significant relative warp axes (Figure 5). Descriptions of the overall shape variations in the dorsal shell portion as described by the relative warps were shown in Table 5.

The shapes of the whorls in the topmost portion of the shells of *P. canaliculata* were also observed and described using outlines assigned in the sutures. 199 outline coefficients (199 outlines) were calculated and interpreted using the multivariate Principal Component Analysis (PCA) to identify sources of variation from the whorl shell shape pattern (Torres *et al* 2011). Figure 6 illustrates the mean shell shape (top/whorl portion) of the Golden apple snail population in varying regions.

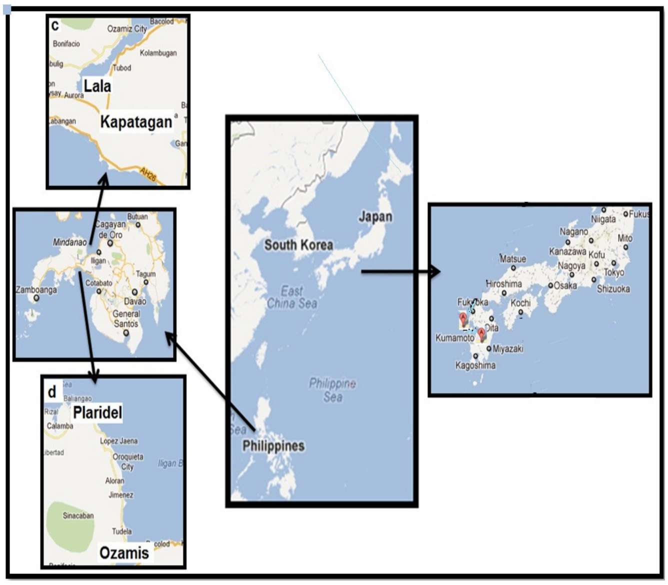

Figure 1. Map showing the study area. (a) Kikuchi, Japan, (b) Okishi, Japan, (c) Lanao del Norte and (d) Misamis Occidental, Philippines.

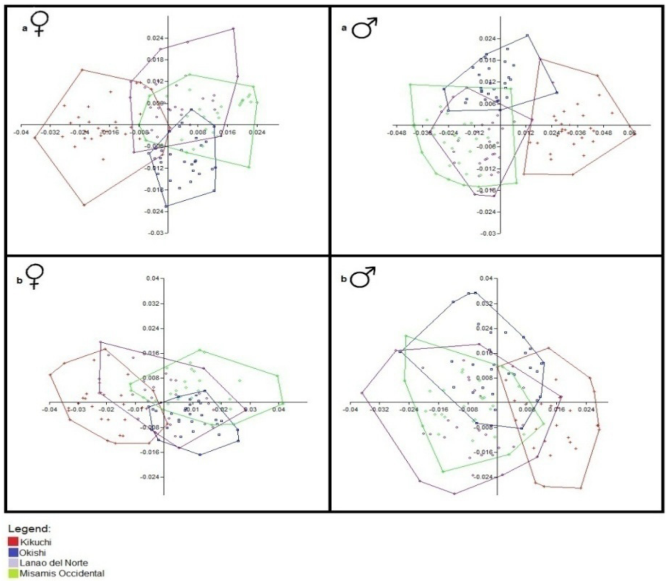

Figure 3. CVA scatter plot of the (a) apertural and (b) dorsal shell of the freshwater snail, *P. canaliculata* showing the female (♀) and male (♂) individuals.

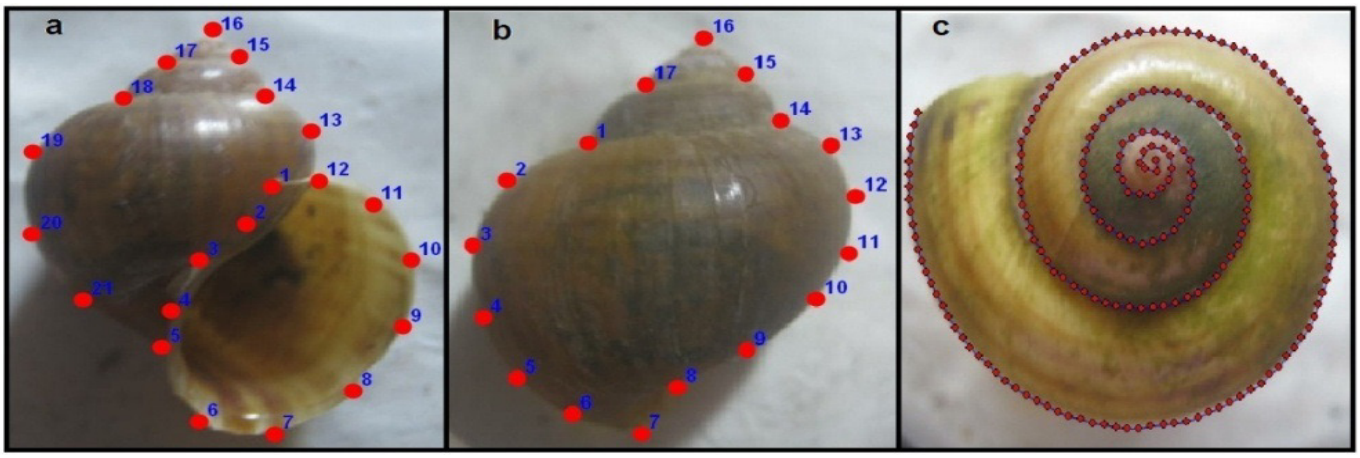

Figure 2. Landmarks used to describe the shape of the (a) ventral, (b) dorsal, and (c) top view of the shell of *Pomacea canaliculata*

Table 1. Results for the comparison of male vs. female data multivariate analysis of variance (MANOVA) p-values

	Ventral/aperture	Dorsal
Female (p-value)	$6.425E^{-22}$	$1.00E^{-13}$
Male (p-value)	$2.768E^{-22}$	$1.561E^{-12}$

The first and second principal components (PC1 and PC2) provide a good summary of the variation for the top/whorl portion of the shell. Results in Table 6 showed the percentage variance value of the significant components in the top/whorl portion of the *P. canaliculata* shell.

To integrate all the three characters in order to observe underlying differences and sources of variability among groups in terms of congruence among characters, Correlation analysis based on distances (CORIANDIS) was also applied (Abdi *et al* 2005, 2007; Tabugo *et al* 2010). Using this analysis, the ventral/apertural, dorsal, and top/whorl portion of the shell were analyzed. Figure 6 is a plot of the principal components of "compromise" space axis accounting for 34.63%, 17.85%, 14.76%, 11.27%, 8.375%, 6.816%, and 6.299% of the total compromise variance. The quality of the compromise is 83.36%.

The congruence and multivariate measure on how related the interspecific locations of traits/characteristics (represented as colored points) are represented in Figure 7. The locations of the populations in the "compromise" space as indicated by the central label reflecting the overall similarity between them as implied by the three shape datasets. Similarly, the intra- and interspecific locations of the three datasets are indicated by the other different points radiating from the "population" points shown at the center.

If the traits tend to be consistently different or similar between pairs of species, they are said to be (positively) congruent, and will show in this plot as a general tendency to cluster together (Tabugo *et al* 2010; Gutierrez *et al* 2011). The locations of the species in the "compromise" space shows a low congruence of the three characters (ventral/aperture, dorsal, and top/whorl) in female and male samples of *P. canaliculata* in Okishi.

Table 2. Results of the Kruskal-Wallis test for significant differences in the mean shapes of ventral/apertural shell of *P. canaliculata*

Relative Warp	Species	a	b	c	d	e	f	g	h
1	a		0.8073	**0.00497**	**$2.38E^{-7}$**	**0.01501**	**0.00172**	**$1.64E^{-5}$**	**$2.68E^{-7}$**
	b	1		**0.00090**	**$1.20E^{-8}$**	**0.00849**	**0.00046**	**$5.86E^{-6}$**	**$3.65E^{-8}$**
	c	0.1391	**0.02529**		**0.00030**	0.8766	0.5298	**0.0228**	**$8.66E^{-5}$**
	d	**$6.66E^{-6}$**	**$3.37E^{-7}$**	**0.00842**		**0.0276**	**0.02976**	0.2871	0.234
	e	0.4204	0.2379	1	0.7729		0.6361	0.1413	**0.00596**
	f	**0.04823**	**0.01284**	1	0.8332	1		0.1984	**0.00666**
	g	**0.00045**	**0.00016**	0.6383	1	1	1		0.0978
	h	**$7.49E^{-6}$**	**$1.02E^{-6}$**	**0.00243**	1	0.1669	0.1867	1	
Relative Warp	**Species**	**a**	**b**	**c**	**d**	**e**	**f**	**g**	**h**
2	a		**$3.01E^{-7}$**	**0.01171**	**0.04133**	0.5395	0.5395	0.1008	0.2198
	b	**$8.43E^{-6}$**		**$4.18E^{-9}$**	**0.00024**	**$1.67E^{-6}$**	**$8.20E^{-7}$**	**$6.53E^{-8}$**	**$1.47E^{-7}$**
	c	0.3279	**$1.17E^{-7}$**		**$2.77E^{-5}$**	0.1669	0.1393	0.5895	0.3711
	d	1	**0.006688**	**0.00078**		**0.0237**	**0.01564**	**0.00100**	**0.00376**
	e	1	**$4.67E^{-5}$**	1	0.6635		0.813	0.4464	0.7062
	f	1	**$2.30E^{-5}$**	1	0.4379	1		0.3953	0.6152
	g	1	**$1.83E^{-6}$**	**1**	**0.0281**	1	1		0.6414
	h	1	**$4.13E^{-6}$**	1	0.1052	1	1	1	
Relative Warp	**Species**	**a**	**b**	**c**	**d**	**e**	**f**	**g**	**h**
3	a		0.61	**0.00433**	0.3292	0.9411	0.05555	0.1761	**0.03205**
	b	1		**0.02028**	0.9	0.4643	**0.01596**	**0.04059**	**0.01221**
	c	0.1211	0.5678		**0.00624**	**0.00081**	**$2.08E^{-6}$**	**$2.68E^{-6}$**	**$6.05E^{-7}$**
	d	1	1	0.1746		0.1785	**0.00081**	**0.01054**	**0.00031**
	e	1	1	**0.02274**	1		**0.04207**	0.1882	**0.013**
	f	1	0.4468	**$5.82E^{-5}$**	**0.02274**	1		0.4464	0.6574
	g	1	1	**$7.50E^{-5}$**	0.295	1	1		0.2772
	h	0.8975	0.3419	**$1.69E^{-5}$**	**0.00866**	0.364	1	1	
Relative Warp	**Species**	**a**	**b**	**c**	**d**	**e**	**f**	**g**	**h**
4	a		0.126	0.947	0.525	0.2772	0.1494	0.5493	0.09049
	b	1		**0.02976**	0.1858	**0.00168**	**0.00081**	**0.00486**	**0.00014**
	c	1	0.8332		0.4825	0.2036	0.07483	0.4333	**0.02866**
	d	1	1	1		0.05844	**0.01695**	0.09334	**0.001767**
	e	1	**0.04703**	1	1		0.5692	0.5592	0.3329
	f	1	**0.02274**	1	0.4747	1		0.3366	0.745
	g	1	0.136	1	1	1	1		0.085
	h	1	**0.00394**	0.8026	**0.04946**	1	1	1	
Relative Warp	**Species**	**a**	**b**	**c**	**d**	**e**	**f**	**g**	**h**
5	a		**0.00814**	**$3.97E^{-8}$**	**$6.1E^{-10}$**	**$1.13E^{-5}$**	**$2.13E^{-5}$**	**$4.99E^{-7}$**	**$3.45E^{-6}$**
	b	0.2278		**0.00269**	**$1.02E^{-5}$**	0.1242	**0.04841**	**0.00282**	0.05188
	c	**$1.11E^{-6}$**	0.07529		**0.01801**	**0.03387**	0.3292	0.9293	0.09334
	d	**$1.71E^{-8}$**	**0.00029**	0.5041		**$5.61E^{-5}$**	**0.00216**	**0.04676**	**0.00013**
	e	**0.00031**	1	0.9485	**0.00157**		0.2772	0.07604	0.5298
	f	**0.000597**	1	1	0.06039	1		0.3147	0.5493
	g	**$1.40E^{-5}$**	0.07903	1	1	1	1		0.1474
	h	**$9.65E^{-5}$**	1	1	**0.0036**	1	1	1	

Legend: Kikuchi, Japan (a) female (b) male, Okishi, Japan (c) female (d) male, Lanao del Norte, Phil. (e) female (f) male, Misamis Occidental, Phil. (g) female (h) male.

Table 3. Percentage variance and overall shape variation in the ventral/apertural shell of golden apple snails as explained by significant relative warps

RW	% variation	Ventral/apertural shell
1	37.72%	A high positive RW1 score means that a shell has less pronounced and narrower shell opening in contrast to the more globose shell opening of the samples along low negative RW1 score. All the golden apple snail populations in varying regions are at either positive or negative RW1 axis signifying that these populations have either wide or narrow aperture.
2	18.73%	Samples along the negative RW2 axis displayed a depressed suture linkage. Male samples from Kikuchi, Japan and Okishi, Japan are observed to draw towards the positive RW2 axis, which separates them from other *P. canaliculata* population. Results of the Kruskal-Wallis test shown in Table 1 have shown significant differences of these populations (sample b and d) to other *P. canaliculata* populations.
3	8.53%	A low negative RW3 score has shell with broader opening and the anterior portion of the inner lip depressed towards the center of the shell opening. On the other hand, a sample with a high positive RW3 score has shorter and narrower shell opening.
4	8.13%	Relative warp axis four illustrates distinction in the height of the spires located in the apical portion of the shell. Samples along the positive axis have a highly elevated spire compared to samples the along negative RW4 axis, which has shorter spires. It was observed that the populations are at either the positive and negative RW4 axis which suggests that the *P. canaliculata* populations in varying regions have either elevated or short spire.
5	8.54%	Lastly, the fifth relative warp explains the differences in the apical and basal shell shapes. A high positive RW5 axis indicates that the shell has a pointed apical and basal portion. While shells on the negative RW5 axis has a depressed apical and basal portion, and the anterior portion of the inner lip depressed towards the center of the shell opening. Female samples from Kikuchi, Japan (sample a) possessed these characteristics which separates it from other *P. canaliculata* population.

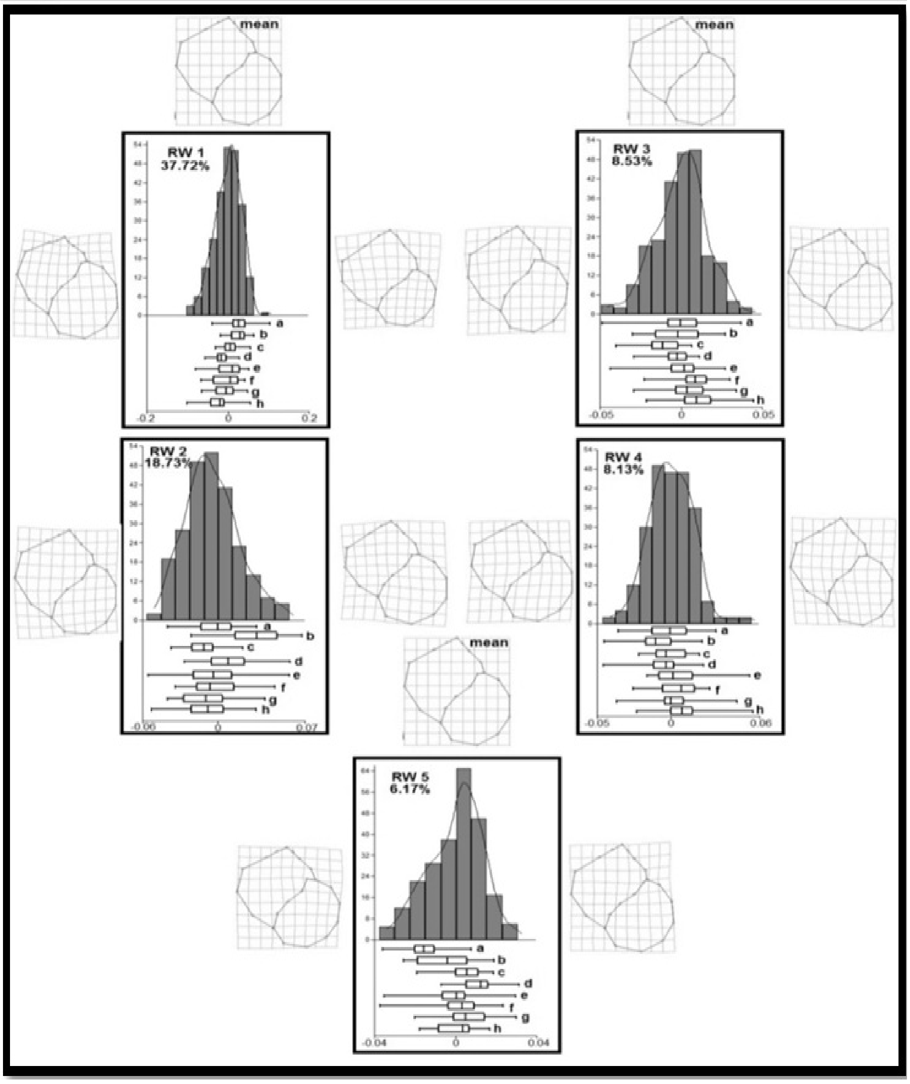

Figure 4. Relative warp box plot and histogram showing variations in the shape of ventral/aperture portion shell of *Pomacea canaliculata* found in varying regions: Kikuchi, Japan (a) female (b) male, Okishi, Japan (c) female (d) male, Lanao del Norte, Phil. (e) female (f) male, Misamis Occidental, Phil. (g) female (h) male.

Table 4. Results of the Kruskal-Wallis test for significant differences in the mean shapes of dorsal shell of *P. canaliculata*

Relative Warp	Species	a	b	c	d	e	f	g	h
1	**a**	0	0.4553	**$1.85E^{-8}$**	**$5.53E^{-8}$**	**0.00181**	**0.00334**	**$7.89E^{-7}$**	**$3.66E^{-7}$**
	b	1	0	**$1.29E^{-6}$**	**$3.09E^{-6}$**	**0.01564**	**0.02236**	**$6.56E^{-5}$**	**$9.51E^{-6}$**
	c	**$5.2E^{-7}$**	**$3.60E^{-5}$**	0	0.8534	**0.01837**	**0.02866**	0.3292	0.8766
	d	**$1.5E^{-6}$**	**$8.66E^{-5}$**	1	**0**	**0.02151**	**0.0345**	0.3953	0.7901
	e	0.05072	0.4379	0.5143	0.6022	0	0.8883	0.1474	**0.02109**
	f	0.09348	0.6261	0.8026	0.966	1	0	0.126	**0.02559**
	g	**$2.2E^{-5}$**	**0.00184**	1	1	1	1	0	0.3007
	h	**$1.0E^{-5}$**	**0.00027**	1	1	0.5905	0.7164	1	0
Relative Warp	**Species**	**a**	**b**	**c**	**d**	**e**	**f**	**g**	**h**
2	**a**	**0**	**$8.29E^{-6}$**	**0.00745**	**$4.08E^{-5}$**	0.3478	0.8418	0.9882	0.6361
	b	**0.0002**	**0**	**0.009069**	0.7675	**0.001174**	**0.000125**	**$1.34E^{-5}$**	**0.000189**
	c	0.2086	0.2539	**0**	**0.03147**	0.1537	**0.03644**	**0.02709**	0.07483
	d	**0.0011**	1	0.881	**0**	**0.00205**	**0.00045**	**0.000133**	**0.000556**
	e	1	**0.03287**	1	**0.05747**	0	0.5011	0.5011	0.8592
	f	1	**0.003494**	1	**0.01249**	1	0	0.9646	0.61
	g	1	**0.000374**	0.7584	**0.00371**	1	1	0	0.7506
	h	1	**0.005297**	1	**0.01557**	1	1	1	0
Relative Warp	**Species**	**a**	**b**	**c**	**d**	**e**	**f**	**g**	**h**
3	**a**	**0**	**0.00210**	**0.00159**	**$2.49E^{-6}$**	**0.00238**	**0.0251**	**$3.83E^{-5}$**	**0.00227**
	b	0.05891	0	0.7062	0.09626	0.7675	0.2707	0.2282	0.7562
	c	**0.0447**	1	0	**0.02236**	0.9941	0.4035	0.09626	0.7338
	d	**$6.9E^{-5}$**	1	0.6261	**0**	**0.02709**	**0.004226**	0.7062	0.05746
	e	0.06664	1	1	0.7584	0	0.4598	0.1023	0.745
	f	0.7028	1	1	0.1183	1	0	**0.01695**	0.3147
	g	**0.0010**	1	1	1	1	0.4747	0	0.1882
	h	0.06344	1	1	1	1	1	1	0

Legend: Kikuchi, Japan (a) female (b) male, Okishi, Japan (c) female (d) male, Lanao del Norte, Phil. (e) female (f) male, Misamis Occidental, Phil. (g) female (h) male.

Table 5. Percentage variance and overall shape variation in the dorsal shell of golden apple snails as explained by significant relative warps

RW	% variation	Dorsal shell
1	49.47%	The first relative warp illustrates the differences in the height of the spire and the shell. Samples found in the positive RW1 axis have the characteristics of a short but more pronounced apical spire. On the other hand, a more elevated but less pronounced apical spire was illustrated on the negative RW1 axis. In terms of the shell height, samples on the low negative RW1 scores have a much shorter shell compared to those with high positive scores. Based on the box plot shown in Figure 5, it was revealed that samples from Kikuchi, Japan are towards the negative axis, while samples from Okishi, Japan are observed to be on the positive RW1 axis. This suggests that samples from Kikuchi have shorter shells and more elevated but less pronounced apical spires, and samples from Okishi have long shells and shorter but more pronounced spires. Moreover, samples from the Philippines are at either positive or negative axis signifying that these samples are either short or long and are either have shorter or elevated whorls.
2	20.53%	Second relative warp axis, which describes the distinction in the basal portion of the dorsal shell in the lower outer lip.
3	10.03%	A high positive RW3 score means that the shell has a thinner width relative to its narrower body whorl. On the other hand, samples in the low negative RW3 axis have shells with wider body whorl and thicker width leading to much shorter spire.
4	6.57%	All *P. canaliculata* samples in varying regions are at either the positive or negative RW4 axis.

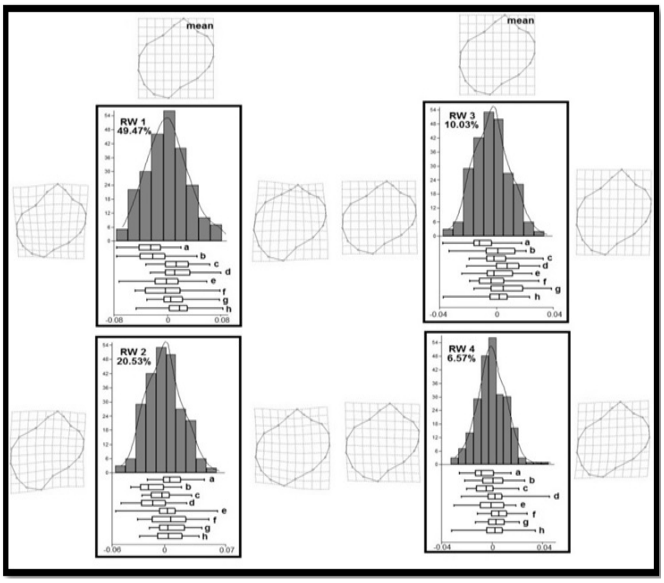

Figure 5. Relative warp box plot and histogram showing variations in the shape of dorsal shell portion of *Pomacea canaliculata* found in varying regions: Kikuchi, Japan (a) female (b) male, Okishi, Japan, (c) female (d) male, Lanao del Norte, Phil. (e) female (f) male, Misamis Occidental, Phil. (g) female (h) male

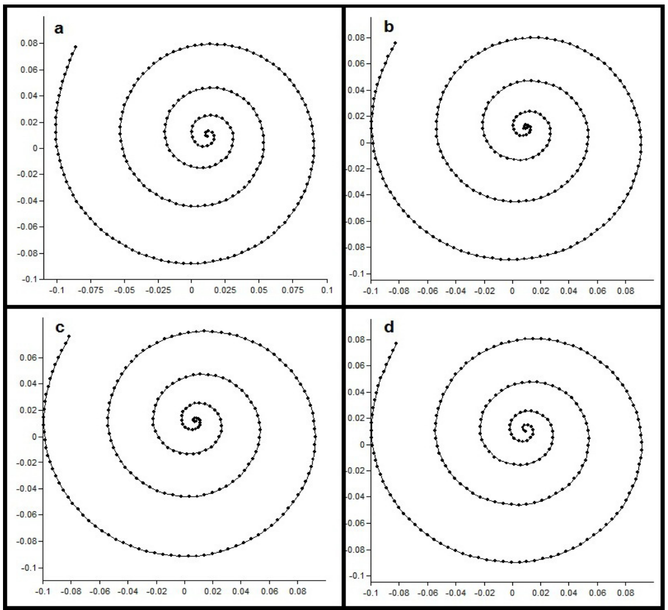

Figure 6. Principal deformations from the mean shape of the top/whorl shell portion of P. canaliculata in varying regions: (a) Kikuchi, Japan, (b) Okishi, Japan, (c) Lanao del Norte, Phil., and (d) Misamis Occidental, Phil.

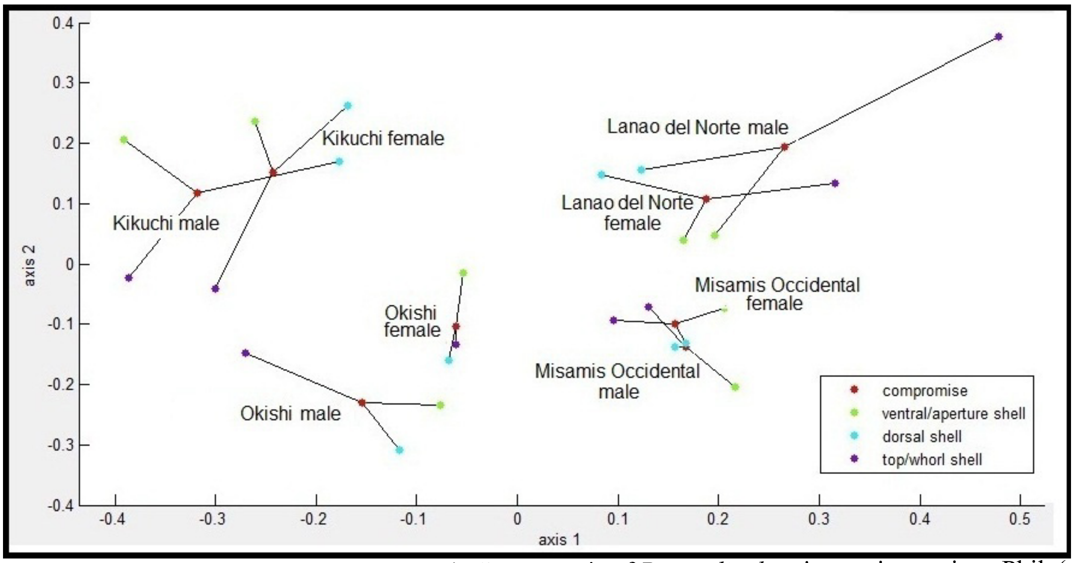

Figure 7. Plot of the principal components of "compromise" space axis of *P. canaliculata* in varying regions.Phil. (e) female (f) male, Misamis Occidental, Phil. (g) female (h) male

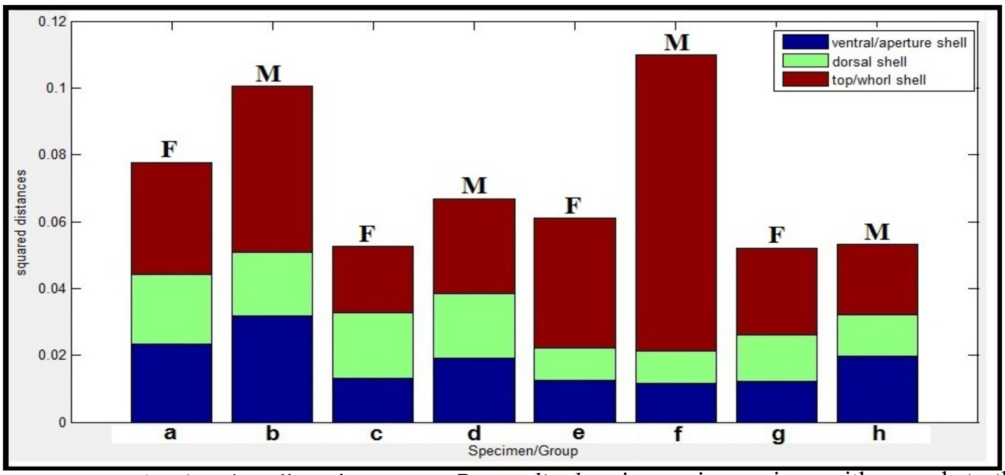

Figure 8. Stacked bar graphs showing disparity among *P. canaliculata* in varying regions with regards to the shape of the ventral/aperture, dorsal, and top/whorl shell portion. Legend: Kikuchi, Japan (a) female (b) male, Okishi, Japan, (c) female (d) male, Lanao del Norte, Phil. (e) female (f) male, Misamis Occidental, Phil. (g) female (h) male

Table 6. Percentage variance values of the significant components in the top/whorl portion of the P. canaliculata shell in varying regions

	JAPAN				PHILIPPINES			
	Kikuchi		**Okishi**		**Lanao del Norte**		**MisamisOccidental**	
PC	**EV**	**V (%)**	**EV**	**V (%)**	**EV**	**V (%)**	**EV**	**V (%)**
PC1	0.015361	77.677	0.02113	79.773	0.02355	78.413	0.017707	75.613
PC 2	0.001487	7.5186	0.00218	8.2161	0.00298	9.917	0.002194	9.373
Total		85.196		87.989	88.33		84.986	84,986

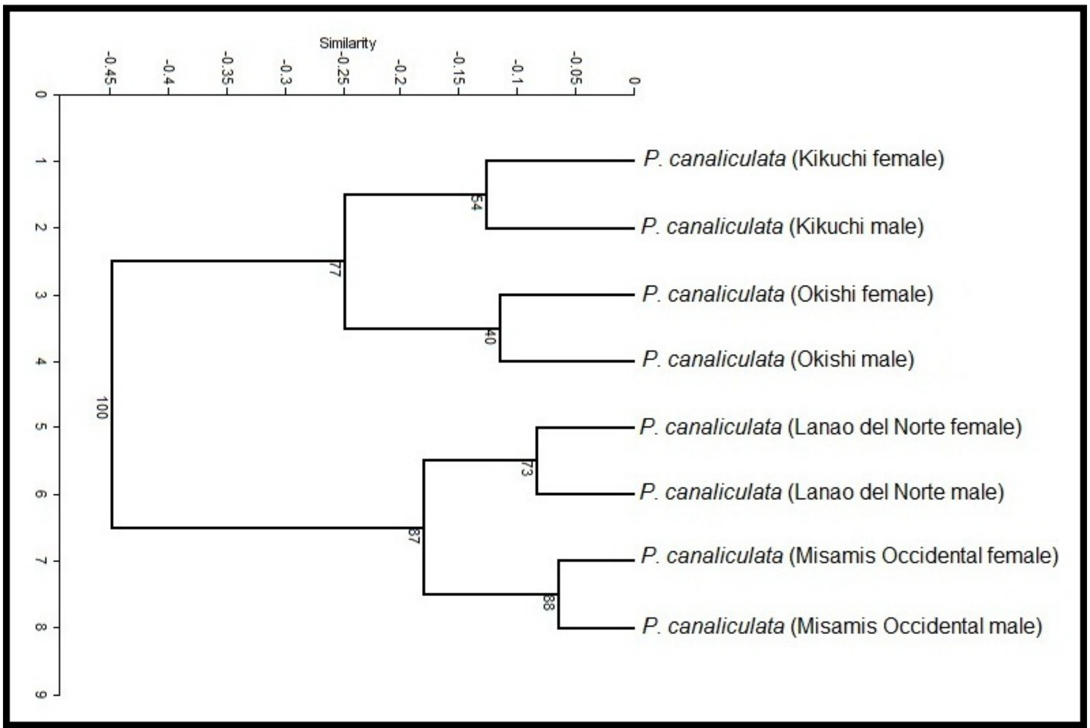

Figure 9. Plot showing the degree of similarity of shell characters of *P. canaliculata* found in varying regions

However, characteristics shown in Kikuchi, Lanao del Norte, and Misamis Occidental samples for each sex illustrates congruence signifying no observable differences in the average shapes between two sexes per population. In addition, the results showed a general tendency for each population to cluster out together implying distinction of *P. canaliculata* populations in varying regions, which suggests geographical variation of the species.

Figure 8 is a stacked bar graphs showing disparity among populations of golden apple snails with regards to the shape of the ventral/aperture, dorsal, and top/whorl portion of the shell. The total height of the stacked bar chart results from the addition of the squared distances of each trait separately (a measure of trait disparity), this shows how much each population differs from the rest by interpreting such differences in terms of individual character (Gutierrez *et al* 2011). This chart shows the level of variation of the three characters exhibited by different golden apple snail populations. Based on the results, samples from Kikuchi and Okishi for each sex showed slightly similar structures in the dorsal portion of the shell. However, these populations are highly variable in the aperture and top/whorl shell as shown by the height of the stacked bar. Additionally, samples from Lanao del Norte for each sex showed similar structure in the ventral/aperture and dorsal portion the shell. These two populations still vary in the top/whorl portion of the shell. Moreover, samples from Misamis Occidental for each sex illustrate similar dorsal and top/whorl shell shape patterns. Nevertheless, variation in the apertural portion of the shell was also observed.

The overall relationship among the golden apple snails on compromise space is shown as a dendrogram in Fig. 9. Results showed that the golden apple snails are divided into four groups based on the shell characters analyzed. The cluster illustrates distinction among populations from Kikuchi (54% similarity), Okishi (40% similarity), Lanao del Norte (73% similarity), and Misamis Occidental (88% similarity). Moreover, the samples collected in Kikuchi and Okishi, Japan clustered together with 77% similarity. On the other hand, the other cluster is composed of samples collected in Lanao del Norte and Misamis Occidental in Mindanao, Philippines with 87% similarity. The results showed morphological disparity of golden apple snails between samples collected in the Philippines and Japan. Thus, geographically separated populations showed different morphologies suggesting geographical variation of P. canaliculata. Moreover, results show a general tendency for each sex of *P. canaliculata* to cluster together implying minimal sexual dimorphism with regards to the characters being analyzed.

Variation among the population of *P. canaliculata* maybe due to phenotypic plasticity, since snails were taken from different geographical regions, thus different environment made them varied from one another. Environmental effects such as physico-chemical factors and predation factors contribute to phenotypic plasticity (Minton *et al* 2011). In freshwater snails, these changes are often accompanied by a change in spire height and aperture size; high, narrow spires and small apertures may reduce predation in headwaters (Lagesson 2011), while low spires and large apertures may reduce dislodging and damage during tumbling (Haase 2003). Phenotypic plasticity is a great strategy because it allows organisms that cannot change their genes a way of changing their phenotypes to meet the demands of the environment (Hollander & Butlin 2010). Thus, phenotypic plasticity evolved to allow organisms a greater chance of survival in changing environments.

Conclusions

Snail shell morphology is traditionally quantified through straight-line shell measurements and ratios. However, traditional measurements can be strengthened by applying quantitative tools to provide a more reliable descriptive analysis of shell shape. Thus, the use of geometric morphometric analysis must be done to determine the limits of shell shape variation. The results of geometric morphometric analysis show that *P. canaliculata* shell varies in shape, where geography contributes in shaping the structure of the populations. Geographic distance is a factor in contributing to disparity with regards to the shape of the shell when viewed from all aspects (apertural, dorsal and whorl), using Correlation analysis based on distances (CORIANDIS). Similarly, the variations observed among the populations might also indicate phenotypic plasticity in golden apple snails that take advantage of their suitability to varying environments. Conversely, there are no observable differences in the average shapes between sexes per population which strongly suggest minimal sexual dimorphism in P. canaliculata. Variability is the raw material of adaptability and long-term survivability. These are the factors attributable to resistance to several pests, including the serious agricultural rice pest P. canaliculata. Awareness of the variability in the golden apple snails is important if effective management strategies are to be developed. Thus, the general structure of the shell shape variation in this study is informative in the management of P. canaliculata. Moreover, the results of this study showed that applications of geometric morphometrics coupled with CORIANDIS are good tools in elucidating geographic differentiation among the populations of the golden apple snail.

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Authors

•Carlo Stephen O. Moneva, Department of Biological Sciences, College of Science and Mathematics, MSU-Iligan Institute of Technology, Andres Bonifacio Avenue, Tibanga, Iligan City, Lanao del Norte, Philippines, carlox_xia@yahoo.com.

•Mark Anthony J. Torres, Department of Biological Sciences, College of Science and Mathematics, MSU-Iligan Institute of Technology, Andres Bonifacio Avenue, Tibanga, Iligan City, Lanao del Norte, Philippines, torres.markanthony@gmail.com.

•Takashi Wada, Kyushu-Okinawa National Agricultural Research Center, Suya 2421, Kohshi-shi, Kumamoto 861-1192, Japan, twada@affrc.go.jp

•Ravindra Joshi, Philippine Rice Research Institute (PHILRICE), Science City of Munoz, Nueva Ecija, Philippines, joshiraviph@gmail.com, joshiraviph@yahoo.com, rcjoshi@philrice.gov.ph

•Cesar G. Demayo, Department of Biological Sciences, College of Science and Mathematics, MSU-Iligan Institute of Technology, Andres Bonifacio Avenue, Tibanga, Iligan City, Lanao del Norte, Philippines, cgdemayo@gmail.com.

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